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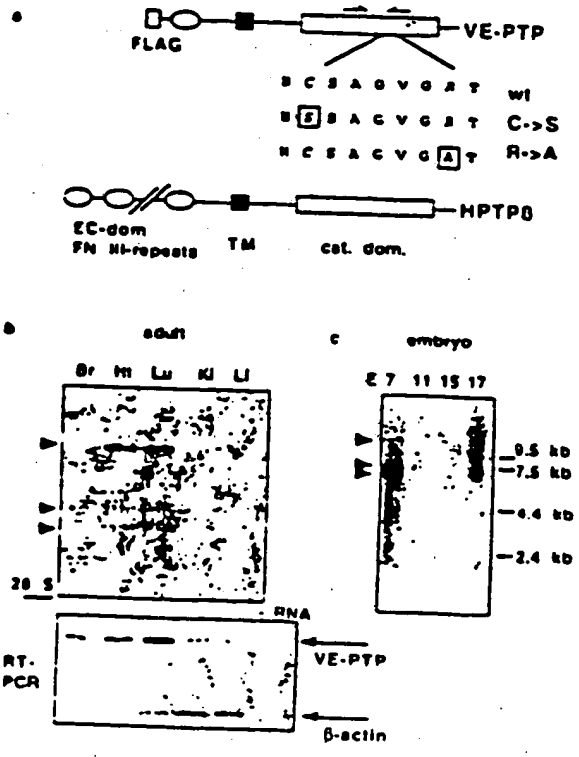
(57) Abstract

Use of vertebrate vascular-endothelial protein tyrosine phosphatases (i.e. murine phosphatase VE-PTP or human phosphatase HPTPβ) or portions thereof for the manufacture of an agent for monitoring or modulating the activity of the endothelial receptor-type tyrosine kinase Tie-2.



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Interaction of vascular-endothelial protein-tyrosine phosphatase with the Angiopoietin receptor Tie-2

Specification

The present invention relates to a method for monitoring or modulating the activity of the angiopoietin receptor-type tyrosine kinase Tie-2.

A key mechanism in the proliferation and differentiation control of all cells are membrane-located receptors, whose activation in many cases is mediated by external factors via phosphorylation of tyrosine residues. The mutation of a series of endothelial cell specific receptor-tyrosine kinases (RTKs) results in lethal phenotypes early during murine embryonal development (Hanahan, Science 277 (1997), 48 - 50; Risau, Nature 386 (1997), 671 - 674). The proliferation and differentiation of endothelial cells depends on two receptor tyrosine kinase systems. The vascular endothelial growth factor (VEGF) is a secreted angiogenic factor and promotes vascularization by activation of its high affinity receptors VEGFR-1 (Flt-1) or VEGFR-2 (Flk-1). The RTKs Tie-1 and Tie-2 are involved in the sprouting and remodelling of the embryonic vascular system. The activity of these kinases is regulated by the recently identified ligands, the angiopoietins.

After ligand binding RTKs are activated by phosphorylation on tyrosine residues. Specific protein-tyrosine phosphatases (PTPs) are involved in the fine-tuning of RTK activity. Several classes of PTPs have been identified. However, the biological functions thereof are presently not understood (Neel & Tonks, Curr. Opin. Cell Biol. 9 (1997), 193 - 204; Streuli, Curr. Opin. Cell Biol. 8 (1996), 182 - 188).

In a study to identify PTPs in endothelial cells a murine vascular-endothelial protein-tyrosine phosphatase VE-PTP was identified (VE-PTP: a receptor

protein-tyrosine phosphatase expressed in vascular endothelium, EMBO-FEBS Workshop on Protein Phosphatases and Protein Dephosphorylation, Oxford, UK, September 21 - 26, 1997). Indications for a functional interaction between VE-PTP and a receptor-type kinase have not been described, however. Further, the association of PTPs with their substrates is difficult to determine due to the transcient nature of the enzyme substrate association (Flint et al., Proc. Natl. Acad. Sci. U.S.A. 94 (1997), 1680 - 1685).

The experiments underlying the present application discovered that VE-PTP is a homolog of the human HPTPβ (Krueger et al., EMBO J., 9, (1990), 3241 - 3252), and that it is specifically expressed in endothelial cells both during the embryonal development of mice and in brain capillary vessels of newborn animals. Biochemical analyses using VE-PTP trapping mutants show a specific interaction between the C-terminal part of the molecule which contains the catalytic domain and the RTK Tie-2 but not with the vascular endothelial growth factor receptor VEGFR-2. Moreover, a dephosphorylation of Tie-2 could be detected in the presence of VE-PTP in transiently transfected COS-1 cells. These data identify Tie-2 as a specific substrate for VE-PTP and show that it is a specific modulator of Tie-2 activity.

This result is of high clinical relevance, as Tie-2 holds a key position in angiogenetic processes, the formation of the blood vessel system during embryonal development, the healing of wounds as well as in pathological processes, e.g. tumor development. As VE-PTP shows a specific interaction with Tie-2 and can modulate the tyrosine phosphorylation of the latter, the receptor-protein tyrosine phosphatase is a target both for diagnostic monitoring and for therapeutically influencing the said processes.

Thus, a subject matter of the present invention is the use of vertebrate, e.g. mammalian vascular-endothelial protein-tyrosine phosphatases or portions

thereof for the manufactur of an agent for monitoring or modulating the activity of receptor-type tyrosine kinase Tie-2.

A further subject matter of the present invention is the use of nucleic acids encoding vertebrate, e.g. mammalian vascular-endothelial protein-tyrosine phosphatases or portions thereof for the manufacture of an agent for monitoring or modulating the activity of receptor-type tyrosine kinase Tie-2.

Still a further subject matter of the invention is the use of ligands for vertebrate, e.g. mammalian vascular-endothelial protein-tyrosine phosphatases for the manufacture of an agent for monitoring or modulating the activity of receptor-type tyrosine kinase Tie-2.

The vascular-endothelial protein-tyrosine phosphatases and nucleic acids coding therefor, e.g. genes or cDNA molecules, are obtainable from vertebrate cells, preferably from mammalian endothelial cells, e.g. murine or human cells. Preferably the vascular-endothelial protein-tyrosine phosphatase is selected from murine phosphatase VE-PTP, human phosphatase HPTPβ or portions thereof, particularly portions comprising the catalytic domain which is located at the C-terminus of the molecule (Fig. 1a). The nucleic acid sequence and the corresponding amino acid sequence of murine vascular-endothelial protein-tyrosine phosphatase are depicted in SEQ. ID. NO 1 and 2, respectively. The corresponding sequences of the human protein, which were identified by Krueger et al. (supra) are depicted in SEQ. ID. NO 3 and 4.

The polypeptide or a portion thereof is suitable for monitoring or modulating the activity of receptor-type tyrosine kinase Tie-2. In addition to a phosphatase with unmodified sequence of the catalytic domain also mutants thereof, which show a modified, e.g. enhanced binding to Tie-2, e.g. the trapping mutants as depicted in Fig. 2 are suitable for the present

invention. Particularly mutants, which exhibit an enhanced binding to Tie-2 are well suited for diagnostic and therapeutic applications.

The interaction between the vascular endothelial protein-tyrosine phosphatase and its substrate Tie-2 can also be monitored and/or modulated on the nucleic acid level. To this end nucleic acids, e.g. DNA molecules, RNA molecules or artificial nucleic acid analogs such as peptidic nucleic acids may be used. Preferably these nucleic acids comprise at least 15, particularly at least 20 nucleotides from murine phosphatase VE-PTP gene, human phosphatase HPTPβ gene or sequences complementary thereto. These nucleic acids are suitable for the determination of the PTP expression by using known hybridization or/and amplification techniques such as PCR. On the other hand, nucleic acids can be used for the modulation of the VE-PTP expression in the form of antisense constructs or as ribozymes.

A still further aspect of the invention is the use of ligands for vertebrate, e.g. mammalian vascular endothelial-protein tyrosine phosphatases. Examples of such ligands are antibodies, e.g. polyclonal or monoclonal antibodies and antibody fragments. Polyclonal antibodies are available according to known protocols by immunization of test animals with purified VE-PTP, HPTPβ or partial fragments thereof, which preferably contain the catalytic domain. From these test animals monoclonal antibodies can be generated in a known manner by using the method applied by Koehler and Milstein. The polyclonal or monoclonal antibodies can also be used in the form of fragments which are obtainable by proteolyic treatment or genetic engineering.

One embodiment of the invention concerns the monitoring or detection of the Tie-2 activity. This detection can be carried out by using kn wn methods, e.g. using labelled polypeptides, nucleic acids or antibodies. A

further embodiment concerns the modulation of the Tie-2 activity. Thereby a stimulation or a repression of the Tie-2 activity is possible.

Of major importance is the examination or influencing of the interaction between VE-PTP and Tie-2 for angiogenesis. Thus the present invention provides means for inducing or for inhibiting vascular growth or remodelling and blood vessel maturation. Particularly, the present invention provides means for inhibiting tumor growth and formation of tumor metastases, e.g. by repressing Tie-2 activity in target cells.

Moreover, the invention is explained by the following figures and sequence protocols.

Fig. 1a shows the schematic representation of VE-PTP, its genetically engineered trapping mutants and HPTPβ.

Fig. 1b and c show Northern blot and RT-PCR analyses of VE-PTP expression in mouse tissues and during mouse embryonic development.

Fig. 2 shows *in vivo* expression analysis of VE-PTP by *in situ* hybridization.

Fig. 3 shows biochemical interactions of VE-PTP trapping mutants with Tie-2 protein.

Fig. 4 shows selective dephosphorylation of Tie-2, but not VEGFR-2 by wild-type VE-PTP.

Fig. 5 shows a sequence comparison of the C-terminus of HPTPβ with VE-PTP and the translated "mRPTPβ" sequence. Known protein domains are depicted:

Membrane proximal FN III-domain (blue), transmembrane domain (red) and catalytic domain (green). The catalytic center is characterized by a $C(x)_8R$-motif.

SEQ. ID. NO. 1 and 2 show the nucleotide sequence of VE-PTP cDNA and the corresponding amino acid sequence.

SEQ. ID. NO. 3 and 4 show the nucleotide sequence of HPTPβ cDNA and the corresponding amino acid sequence.

Example 1

A PCR screen of a murine brain capillary cDNA library and reverse transcribed mRNA of bEND5 endothelioma cells to identify endothelial specific members of the protein-tyrosine phosphatase family was performed. For PCR, 100 pmol degenerated primers RPTP1 5'-GA(C/T) TT(C/T) TGG ATG (A/G/T) (G/T)I TGG GA-3' and RPTP2 5'-CCI ACI CGI GCI (G/C)(A/T)(A/G) CA(A/G) TGI AC-3' in 50 µl reactions were used. As templates 1.25 µg λ-DNA from mouse P4-10 brain capillary-library (Schnürch & Risau, Development, 119 (1993), 957 - 968) or 3 µl of SuperScript cDNA preparation (GIBCO BRL) from bEND5 mRNA were used. Isolated 370 bp products were cloned into the vector pCRII (Invitrogen), analysed by restriction cleavage and sequenced on an ABI 370 automated sequencer (Applied Biosystems).

One of the identified PCR products encodes a polypeptide, designated as vascular-endothelial protein-tyrosine phosphatase (VE-PTP) which was identified as murine homolog of the previously described receptor-type protein-tyrosine phosphatase HPTPβ (Krueger et al. EMBO J. 9 (1990), 3241 - 3252). VE-PTP and HPTPβ belong to the subclass III f receptor-type PTPs bearing exclusively fibronectin type III-like repeats in the extracellular

domain and a single catalytic domain in the cytoplasmatic tail (Fig. 1a) (Brady-Kalnay & Tonks, Curr. Opin. Cell. Biol. 7 (1995), 650 - 657).

Fig. 1a shows a schematic representation of VE-PTP, its genetically engineered trapping mutants C->S, R->A and HPTPβ. Rectangles indicate mutated amino acids in the catalytic core. The location of the degenerated primers used in the PCR screen are indicated by arrows (EC-dom., extracellular domain; FN III fibronectin-type III-like repeat; cat. dom., catalytic domain).

Example 2

A Northern blot and RT-PCR analysis of VE-PTP expression in mouse tissues and during mouse embryonic development were performed. A 751 bp EcoRI-fragment from VE-PTP part 1, obtained by PCR using primers PrPTP$\beta$for 5'-GGA AGA GGT ACC TGG TGT CCA TCA AGG-3' and PrPTPβrev 5'-GGC CGG TCC CTA CGA ATG CTG AGC CGG GCA G-3' deduced from a partial clone of murine "RPTPβ" (Schepens et al. Mol. Biol. Reports, 16 (1992)), and cloned in the vector pBS KS(+)(Stratagene), was labelled with α^{32}P-dCTP (Amersham Pharmacia Biotech). For Northern blot analysis 20 μg of total RNA from mouse tissues (Chomczynski & Sacchi, Analyt. Biochem. 162 (1987), 156 - 159) were loaded on a formaldehyde containing agarose gel and blotted. A mouse embryo mRNA Northern blot was obtained from Clontech and hybridization was carried out according to manufacturer's instructions. Autoradiography was performed at -70° C for 17 d. For semiquantitative PCR 50 μl reactions containing 2 μl of reverse transcribed cDNA preparations and 20 pmol of primers betaseq2 5'- CCC TCT CCC TTC CTA CCT GG-3' and betarev 5'- GGC CGG TCC CTA CGA ATG CTG AGC CGG GCA GG-3' were used, giving a 416 bp fragment. 30 cycles PCR was optimized to detect 1 fg of VE-PTP plasmid DNA. β-actin RT-PCR was performed as described (Nakajima-Iijima et al, Proc. Natl. Acad. Sci. U.S.A. 82 (1985), 6133 - 6137).

Northern blot analysis of VE-PTP expression revealed a major transcript of approximately 11 kB and two additional transcripts of 7.5 and 6 kB. In the adult mouse VE-PTP mRNA was strongly expressed in brain as well as in lung and heart. Very weak expression was detectable in kidney and liver (Fig. 1b). These data were confirmed by semi-quantitative RT-PCR performed with RNA from these organs (Fig. 1b). During embryonic development VE-PTP was weakly expressed at embryonic day E11, expression increased at E15 reaching a maximum at E17 (Fig. 1c). Strong expression was detected at E7, which may result from expression in contaminating maternal tissue as expression in the placenta was observed by *in situ* hybridization analysis as well.

Example 3

An *in vivo* expression analysis of VE-PTP by *in situ* hybridization to frozen sections of mouse embryonic tissues was carried out. The results are shown in Fig. 2. Fig. 2a is a darkfield image of an E12.5 embryo section hybridized with a VE-PTP antisense probe. (NC: neural crest, DA: dorsal aorta). Fig. 2b is a darkfield image and Fig. 2c is a brightfield image of a higher magnification of the vessel indicated in a (asteriks). Fig. 2d - h are sagittal sections of E15.5 embryos hybridized with antisense VE-PTP probes. Fig. 2d is a darkfield image and Fig. 2e a brightfield image of the lung. Fig. 2f is a darkfield image of the head region. Fig. 2g is an E15.5 embryo section hybridized with a VEGFR-2 antisense probe. Fig. 2h - k are vessels in brain sections of P10 mice hybridized with antisense VE-PTP probes. As templates for *in vitro* transcription pCRII (Invitrogen) VE-PTP-1 (370 bp fragment of VE-PTP coding for protein sequence corresponding to aa 1786 - 1913 in HPTPβ in pCRII) and pBS VE-PTPpart1 were used. Sectioning of mouse embryos and *in situ* hybridization were performed as described (Breier et al, Development, 114 (1992), 521 - 532).

At the earliest timepoint analysed (E9.5), expression was detectable in the endothelial cell layer lining the dorsal aortae. During the subsequent developmental stages VE-PTP expression was increased throughout the developing vascular system (Fig. 2a). Strong hybridization signals were visible in endothelial cells forming blood vessels, whereas no specific signals were detected in blood cells or smooth muscle cells surrounding the vessels (Fig. 2b, c). At E15.5 specific signals were detectable in all organs with highest expression in the lung (Fig. 2d,e). Comparison to serial sections hybridized with an antisense probe to VEGFR-2 (Flk-1) as an endothelial cell marker, confirmed the vascular endothelial specific expression pattern of VE-PTP (Fig. 2 f,g). In contrast to the uniform expression levels of VEGFR-2 in different types of embryonic endothelial cells, VE-PTP was more strongly expressed in endothelial cells lining larger, smooth muscle cell invested vessels than those of small capillaries and veins. On brain sections of newborn mice, specific expression of VE-PTP was detectable in brain capillaries as well as in larger vessels (Fig. 2h-k). No specific signals were visible in neuronal or glial cells.

Example 4

The biochemical interactions of VE-PTP with the receptor tyrosine kinases Tie-2 and VEGFR-2 were investigated using bacterial GST-fusion proteins. The results are shown in Fig. 3.

Fig. 3a demonstrates the results of GST-fusion pull down experiments. GST and GST x VE-PTP R/A fusion protein were incubated with lysates from bEND5 cells. Precipitates were blotted with an anti-Tie-2 antibody and reblotted with an VEGFR-2 specific antibody. (tot. lys.: total lysates of bEND5 cells). pGEX-VE-PTP contains a 1.1 kB 3' part of EST-clone 552065 (Lennon et al., Genomics 33 (1996), 151 -152) coding for the cytoplasmic domain f VE-PTP cloned in pGEX 3T (Amersham Pharmacia Biotech). GST and GST-fusion pr teins were expressed in *E.coli* strain TOP10 essentially

as described (Frangioni & Neel, Anal. Biochem. 210 (1993), 179 - 187). For pull down experiments 10 cm dishes of confluent endothelial cells were pretreated with pervanadate, lysed and incubated with 10 µg of GST-fusion protein prebound to glutathion-sepharose as described before (Jallal et al., J. Biol. Chem. 272 (1997), 12158 - 12163).

Fig. 3b shows co-immunoprecipitation of VE-PTP trapping mutants (C->S, R->A) with Tie-2. COS-1 cells were transfected with FLAG-tagged VE-PTP and trapping mutants together with Tie-2. Immunoprecipitation was performed with anti-FLAG antibody M2. Precipitates were blotted with a Tie-2 specific monoclonal antibody.

pCMV-FLAG VE-PTP wt, C->S and R->A contain cDNA sequences coding for a polypeptide stretch corresponding to aa 1418-1977 in HPTPβ cloned in pCMV-FLAG-1 (Kodak). Trapping mutations C->S and R->A were introduced by PCR mutagenesis using primer Prbetamutcs 5'-TCC GTA GTG CAC TCG AGT GCT GGT GTG-3' and primer Prbetamutra 5'-GCT GGT GTG GGC GCC ACA GGG ACG TTC-3'. COS-1 cells (Gluzman, Cell 23 (1981), 175 - 182) were transfected using the modified calcium phosphate method (Chen & Okayama, Mol. Cell. Biol. 7 (1987), 2745 - 2752). For transfection 10 µg of pCMV-FLAG derivates and 2 µg of expression plasmids coding for the RTKs were used. As control 0.5 µg of EGFP expression plasmid (Clontech) were cotransfected. Cells were harvested after 2 d of expression. Transfection efficiency was evaluated under fluorescent light and was usually between 30 - 70%.

In mixing experiments of endothelial cell lysates and trapping mutants of the VE-PTP catalytic domain fused to GST, we detected interaction with the Tie-2 receptor, while GST alone did not precipitate Tie-2. The interaction was independent of pretreatment with pervanadate. In these assays coprecipitation of VEGFR-2 was never detectable (Fig. 3a).

To test for potential substrate interactions with Tie-2 and VEGFR-2 we coexpressed these RTKs with either a FLAG-tagged version of VE-PTP corresponding to aa 1418-1997 of HPTPβ, or the respective trapping mutants (Fig. 1a). Physical association was analysed by co-immunoprecipitation using an anti-FLAG-antibody and subsequent blotting of the precipitates with antibodies specific for the repective RTK. In this assay the Tie-2 receptor co-precipitated with both trapping mutants of VE-PTP (C-> S, R-> A) (Fig. 3b). The wild type phosphatase failed to precipitate Tie-2 efficiently, even though the receptor was expressed at comparable levels. This reduced association of PTPs *in vitro* with their substrates is due to the transient nature of the enzyme substrate association. Unlike Tie-2, VEGFR-2 could neither be co-immunoprecipitated with VE-PTP nor with one of the trapping mutants, even though VEGFR-2 expression was comparable to that of Tie-2.

Example 5

Finally, the phosphorylation state of RTKs was determined in the presence of VE-PTP. Figure 4 shows dephosphorylation of (a) Tie-2 but not (b) VEGR-2 by wild-type VE-PTP. RTKs were immunoprecipitated with specific antibodies from cotransfected COS-1 cells. Precipitates were blotted with anti-phosphotyrosine antibodies and after stripping reprobed with RTK-specific antibodies.

Tie-2 and VEGFR-2 expression vectors were published previously (Koblizek et al., Curr. Biol. 8 (1997), 529 - 532; Millauer et al., Cell 72 (1993), 835 - 846). Rat monoclonal antibodies against Tie-2 clones 3g1 and 4g8 (Koblizek et al. (1997) supra) and Flk-1 clone 12α1 (Kataoka et al., Devel. Growth Diff. 39 (1997), 729 - 740) were used. Immunoprecipitations were performed with 5 μg of the monoclonal antibodies and immunoblotting with 2 μg/ml. Polyclonal anti-Flk-1 serum 1D3 (Sugen) was used in a 1:5000 dilution. Monoclonal anti-Flag antibody M2 (Kodak), polyclonal antiserum

against Tie-2 (Santa Cruz Biotechnology) and monoclonal mouse antibody against phosphotyrosine PY20 (Transduction Labs) were used according to the manufacturer's instructions. Immunoprecipitations and immunoblotting were performed as described before (Esser et al., J. Cell. Biol. 140 (1998), 947 - 959); Jallal et al., J. Cell. Biol. Chem. 272 (1997), 12158 - 12162).

Immunoprecipitates of VEGFR-2 and Tie-2 co-expressed with either the VE-PTP trapping mutants (C->S, R->A) or wt VE-PTP were blotted with an α-phosphotyrosine-specific antibody and then reprobed with antibody specific for the RTK. Only for Tie-2, changes in the phosporylation status were observed. In the presence of the trapping mutants (C->S, R->A) the receptor was reproducibly more highly phosphorylated than in the controls. This hyperphosphorylation of Tie-2 in the presence of catalytically impaired trapping mutants suggests that physical interaction leads to protection of the receptor from dephosphorylation. In contrast, hypophosphorylation of the Tie-2 receptor was observed in the presence of wt VE-PTP, when compared to vector control (Fig. 4a). No significant changes were detected in the phosphorylation status of VEGFR-2, irrespective of the presence of VE-PTP or its trapping mutants (Fig. 4b). These findings clearly show that Tie-2 is a specific substrate for the endothelial specific phosphatase VE-PTP.

Claims

1. Use of vertebrate vascular-endothelial protein-tyrosine phosphatases or portions thereof for the manufacture of an agent for monitoring or modulating the activity of receptor-type tyrosine kinase Tie-2.

2. The use of claim 1 wherein said phosphatase is selected from murine phosphatase VE-PTP, human phosphatase HPTPβ or portions thereof.

3. The use of claim 1 or 2 wherein said portion comprises the catalytic domain.

4. Use of nucleic acids encoding vertebrate vascular-endothelial protein-tyrosine phosphatases or portions thereof for the manufacture of an agent for monitoring or modulating the activity of receptor-type tyrosine kinase Tie-2.

5. The use of claim 4 wherein said nucleic acid comprises at least 15 nucleotides from murine phosphatase VE-PTP nucleic acid, human phosphatase HPTPβ nucleic acid or sequences complementary thereto.

6. The use of ligands for vertebrate vascular-endothelial protein-tyrosine phosphatases for the manufacture of an agent for monitoring or modulating the activity of receptor-type tyrosine kinase Tie-2.

7. The use of claim 7 wherein said ligands are selected from antibodies and antibody fragments.

8. The use of any one of claims 1 - 7 for detecting Tie-2 activity.

9. The use of any one of claims 1 - 7 for stimulating Tie-2 activity.

10. The use of any one of claims 1 - 7 for repressing Tie-2 activity.

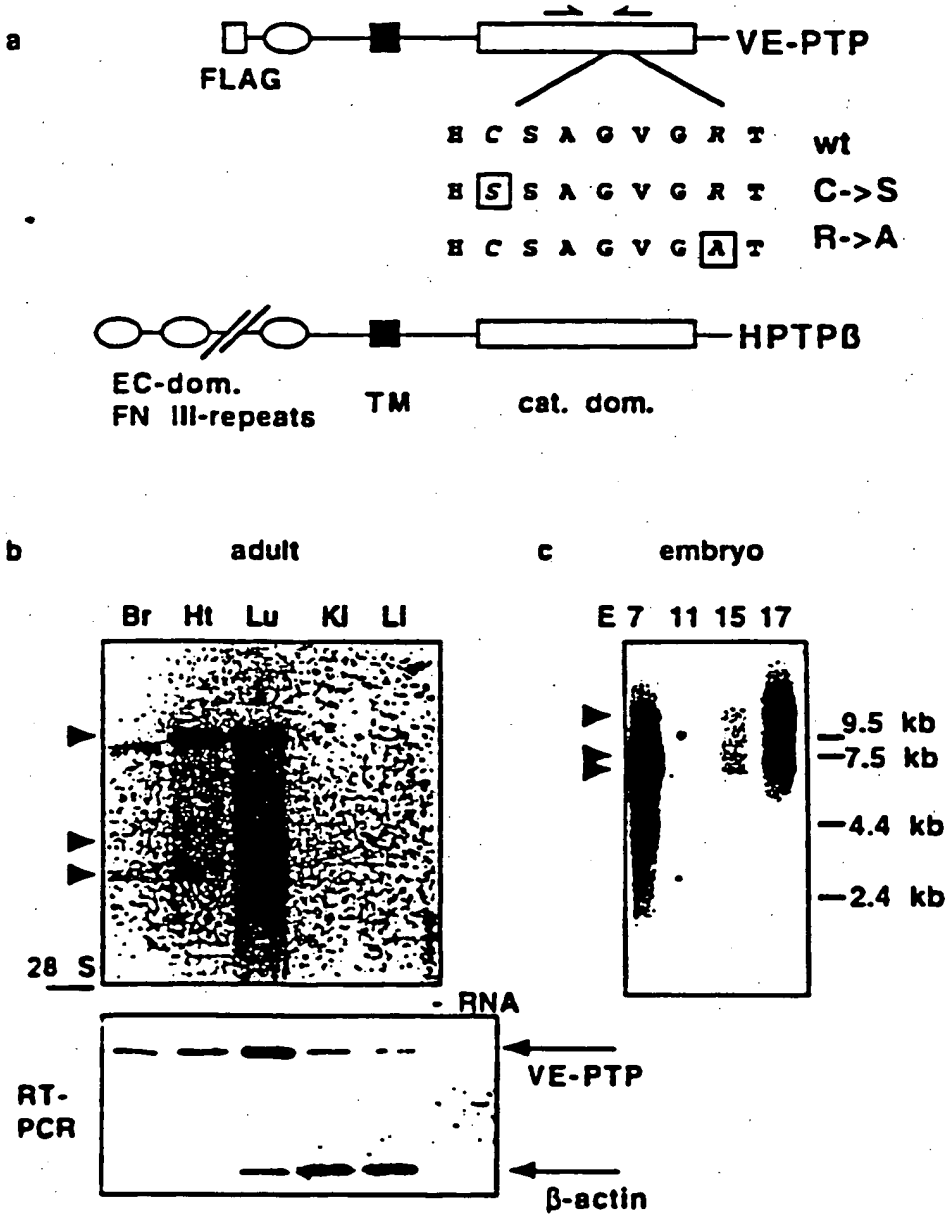
11. The use of any one of the previous claims for monitoring or modulating angiogenesis.

12. The use of any one of the previous claims for inducing vascular growth or remodelling and blood vessel maturation.

13. The use of any one of the previous claims for inhibiting vascular growth or remodelling and blood vessel maturation.

14. The use of any one of the previous claims for inhibiting tumor growth.

15. The use of any one of the previous claims for inhibiting formation of tumor metastases.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FLAG
VE-PTP
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E S S A G V G R T C->S
E C S A G V G A T R->A
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EC-dom.
FN III-repeats
TM
cat. dom.
b
adult
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- RNA
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Fig. 1

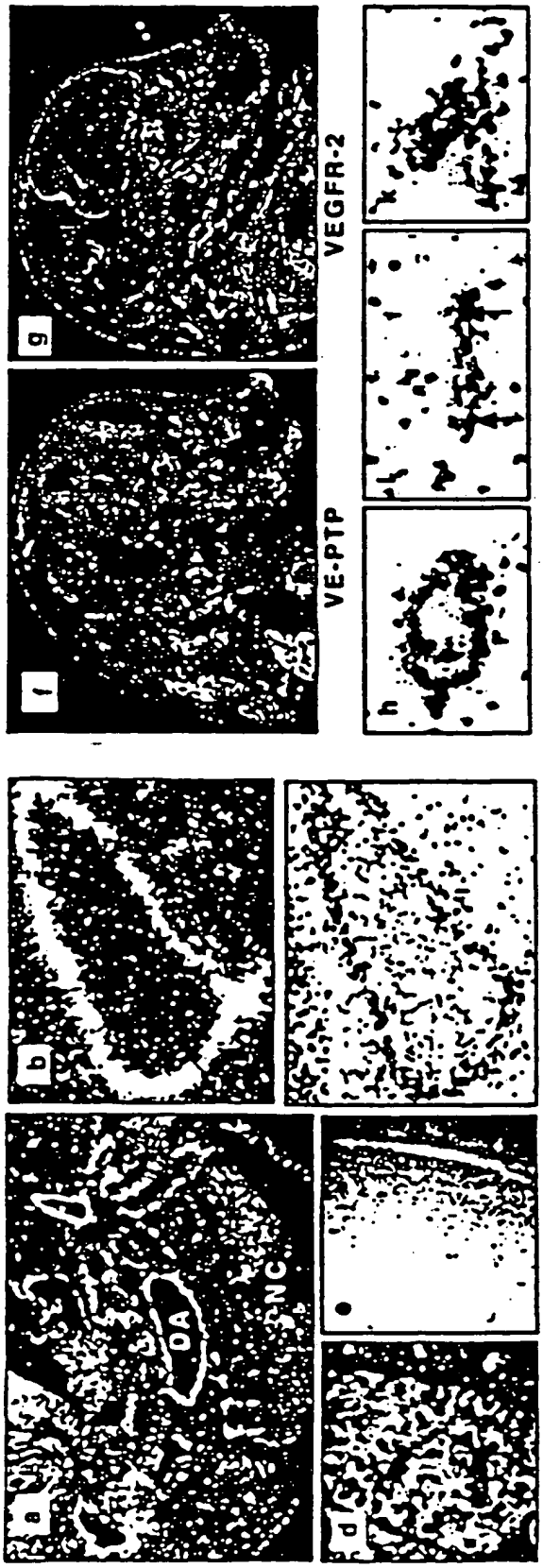
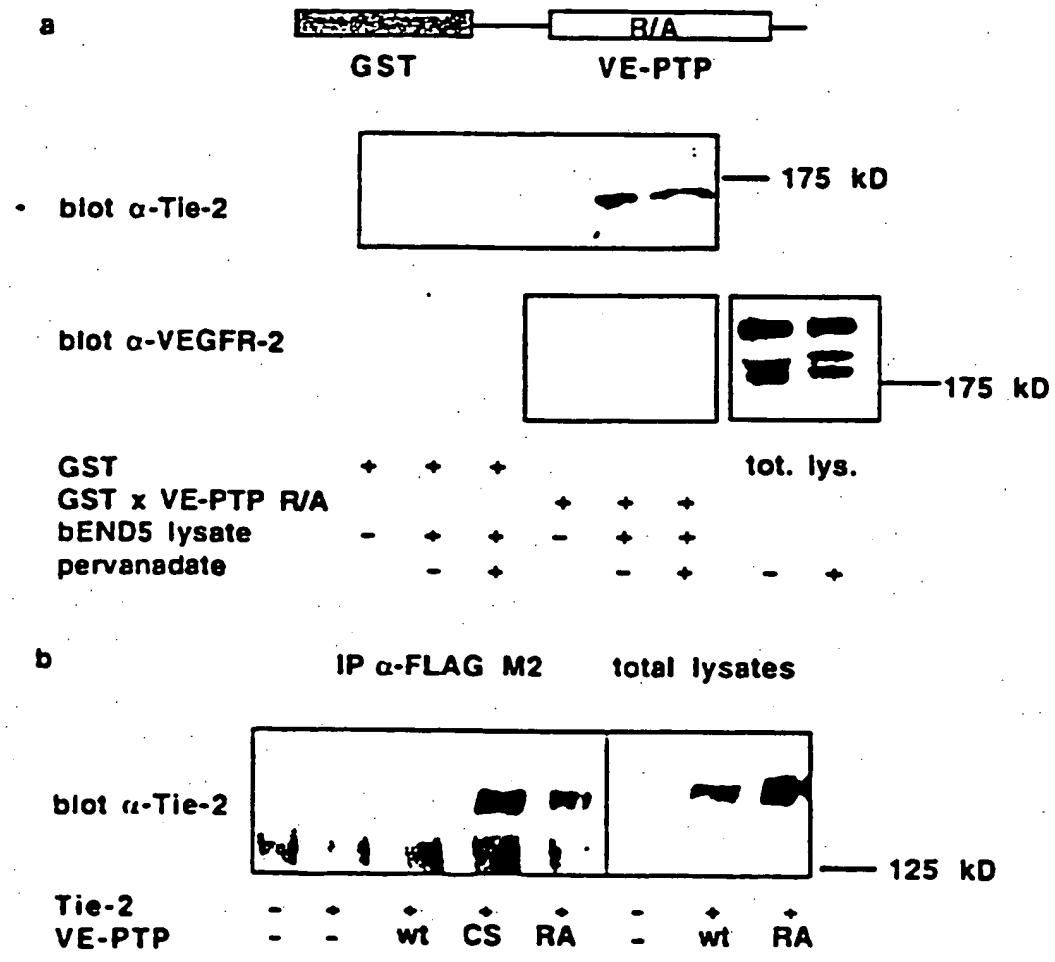


Fig. 2


a
R/A
GST
VE-PTP
blot α-Tie-2
175 kD
blot α-VEGFR-2
175 kD
GST
GST x VE-PTP R/A
bEND5 lysate
pervanadate
tot. lys.
b
IP α-FLAG M2
total lysates
blot α-Tie-2
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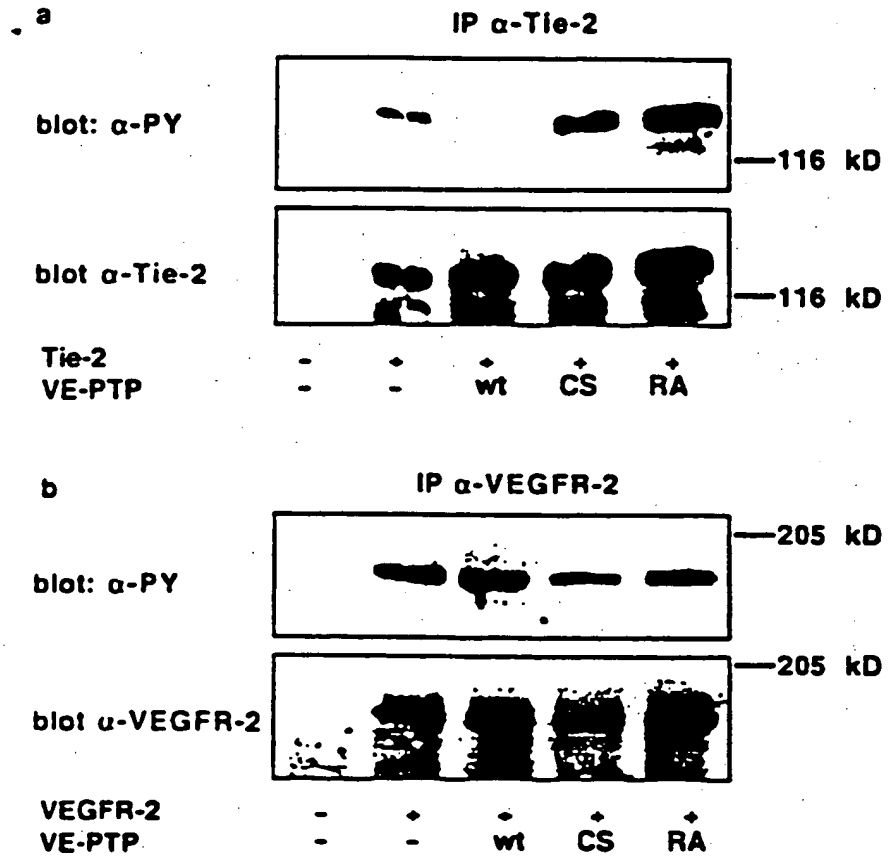
Tie-2
VE-PTP
wt
CS
RA
wt
RA

Fig. 3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blot: α-PY
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blot α-Tie-2
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Tie-2
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blot α-VEGFR-2
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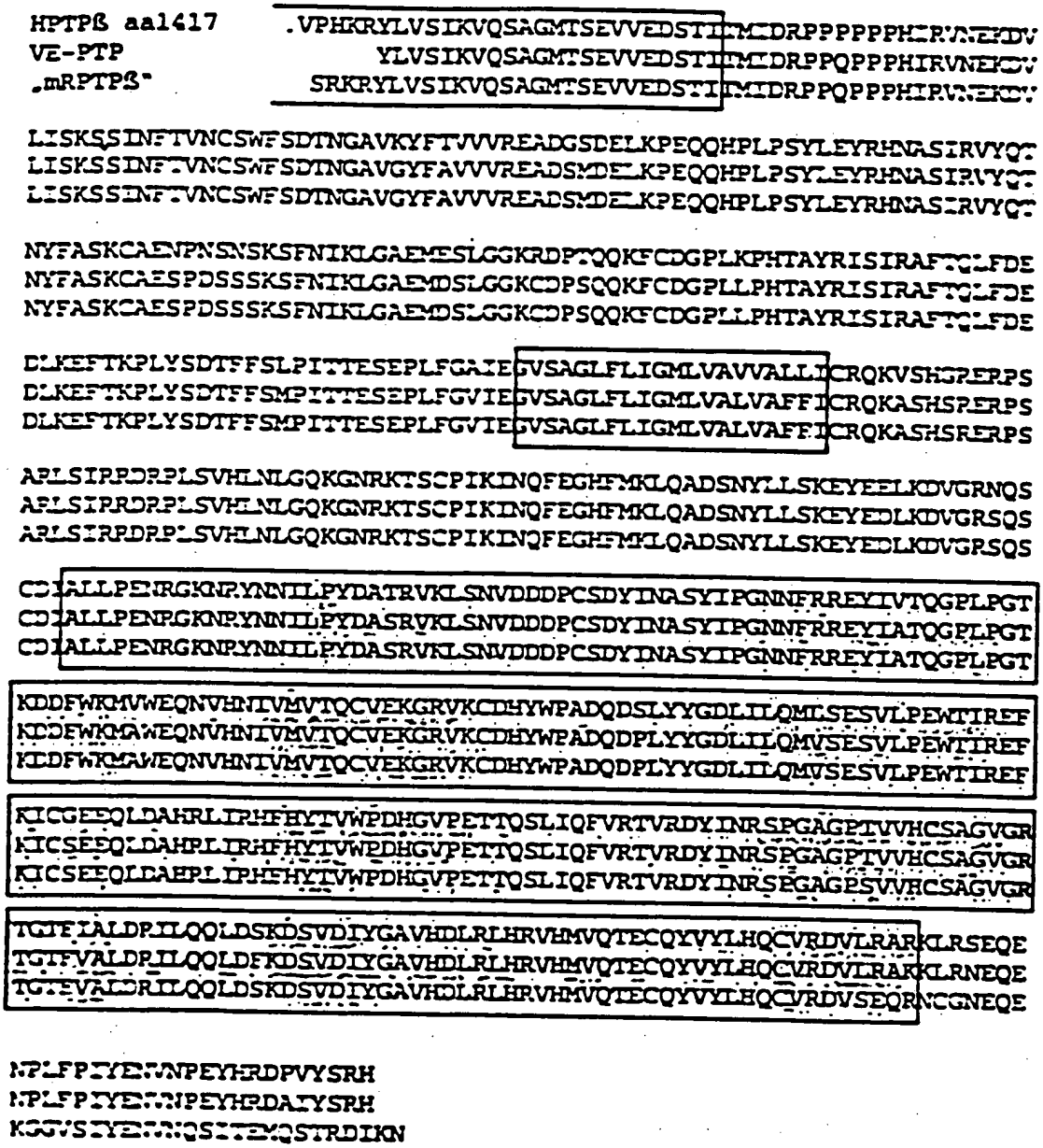
VE-PTP
- - wt CS RA

Fig. 4

Fig. 5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        715                 720                 725

aca gtg cct gac aaa gtc cag gga gtc agt gtt agc aac tca gcc agg   2262
Thr Val Pro Asp Lys Val Gln Gly Val Ser Val Ser Asn Ser Ala Arg
    730                 735                 740

agt gac tat tta agg gta tcc tgg gtg cat gcc act gga gac ttt gat   2310
Ser Asp Tyr Leu Arg Val Ser Trp Val His Ala Thr Gly Asp Phe Asp
745                 750                 755                 760

cac tat gaa gtc acc att aaa aac aaa aac aac ttc att caa act aaa   2358
His Tyr Glu Val Thr Ile Lys Asn Lys Asn Asn Phe Ile Gln Thr Lys
                765                 770                 775

agc att ccc aag tca gaa aac gaa tgt gta ttt gtt cag cta gtc cct   2406
Ser Ile Pro Lys Ser Glu Asn Glu Cys Val Phe Val Gln Leu Val Pro
            780                 785                 790

gta cgg ttg tat agt gtc act gtt act aca aaa agt gga caa tat gaa   2454
Gly Arg Leu Tyr Ser Val Thr Val Thr Thr Lys Ser Gly Gln Tyr Glu
        795                 800                 805

gcc aat gaa caa ggg aat ggg aga aca att cca gag cct gtt aag gat   2502
Ala Asn Glu Gln Gly Asn Gly Arg Thr Ile Pro Glu Pro Val Lys Asp
    810                 815                 820

cta aca ttg cgc aac agg agc act gag gac ttg cat gtg act tgg tca   2550
Leu Thr Leu Arg Asn Arg Ser Thr Glu Asp Leu His Val Thr Trp Ser
825                 830                 835                 840

gta gct aat ggg gat gtc gac caa tat gag atc cag ctg ctc ttc aat   2598
Gly Ala Asn Gly Asp Val Asp Gln Tyr Glu Ile Gln Leu Leu Phe Asn
                845                 850                 855

gac atg aaa gta ttt cct cct ttc cac ctt gta aat acc gca acc gag   2646
Asp Met Lys Val Phe Pro Pro Phe His Leu Val Asn Thr Ala Thr Glu
            860                 865                 870

tat cga ttt act tcc cta aca cca ggc cgc caa tac aaa att ctt gtc   2694
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Tyr Arg Phe Thr Ser Leu Thr Pro Gly Arg Gln Tyr Lys Ile Leu Val
        875                 880                 885

ttg acg att agc ggg gat gta cag cag tca gcc ttc att gag ggc ttc   2742
Leu Thr Ile Ser Gly Asp Val Gln Gln Ser Ala Phe Ile Glu Gly Phe
    890                 895                 900

aca gtt cct agt gct gtc aaa aat att cac att tct ccc aat gga gca   2790
Thr Val Pro Ser Ala Val Lys Asn Ile His Ile Ser Pro Asn Gly Ala
905                 910                 915                 920

aca gat agc ctg acg gtg aac tgg act cct ggt ggg gga gac gtt gat   2838
Thr Asp Ser Leu Thr Val Asn Trp Thr Pro Gly Gly Gly Asp Val Asp
                925                 930                 935

tcc tac acg gtg tcg gca ttc agg cac agt caa aag gtt gac tct cag   2886
Ser Tyr Thr Val Ser Ala Phe Arg His Ser Gln Lys Val Asp Ser Gln
            940                 945                 950

act att ccc aag cac gtc ttt gag cac acg ttc cac aga ctg gag gcc   2934
Thr Ile Pro Lys His Val Phe Glu His Thr Phe His Arg Leu Glu Ala
        955                 960                 965

ggg gag cag tac cag atc atg att gcc tca gtc agc ggg tcc ctg aag   2982
Gly Glu Gln Tyr Gln Ile Met Ile Ala Ser Val Ser Gly Ser Leu Lys
    970                 975                 980

aat cag ata aat gtg gtt ggg cgg aca gtt cca gca tct gtc caa gga   3030
Asn Gln Ile Asn Val Val Gly Arg Thr Val Pro Ala Ser Val Gln Gly
985                 990                 995                 1000

gta att gca gac aat gca tac agc agt tat tcc tta ata gta agt tgg   3078
Val Ile Ala Asp Asn Ala Tyr Ser Ser Tyr Ser Leu Ile Val Ser Trp
                1005                1010                1015

caa aaa gct gct ggt gtg gca gaa aga tat gat atc ctg ctt cta act   3126
Gln Lys Ala Ala Gly Val Ala Glu Arg Tyr Asp Ile Leu Leu Leu Thr
            1020                1025                1030

gaa aat gga atc ctt ctg cgc aac aca tca gag cca gcc acc act aag   3174
Glu Asn Gly Ile Leu Leu Arg Asn Thr Ser Glu Pro Ala Thr Thr Lys
        1035                1040                1045

cac cac aaa ttt gaa gat cta aca cca ggc aag aaa tac aag ata cag   3222
Gln His Lys Phe Glu Asp Leu Thr Pro Gly Lys Lys Tyr Lys Il  Gln
    1050                1055                1060

atc cta act gtc agt gga ggc ctc ttt agc aag gaa gcc cag act gaa   3270
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Ile Leu Thr Val Ser Gly Gly Leu Ph  Ser Lys Glu Ala Gln Thr Glu
1065                1070                1075                1080

ggc cga aca gtc cca gca gct ctc acc gac ctg agg atc aca gag aac   3318
Gly Arg Thr Val Pro Ala Ala Val Thr Asp Leu Arg Ile Thr Glu Asn
                1085                1090                1095

tcc acc agg cac ctg tcc ttc cgc tgg acc gcc tca gag ggg gag ctc   3366
Ser Thr Arg His Leu Ser Phe Arg Trp Thr Ala Ser Glu Gly Glu Leu
            1100                1105                1110

agc tgg tac aac atc ttt ttg tac aac cca gat ggg aat ctc cag gag   3414
Ser Trp Tyr Asn Ile Phe Leu Tyr Asn Pro Asp Gly Asn Leu Gln Glu
        1115                1120                1125

aga gct caa gtt gac cca cta gtc cag agc ttc tct ttc cag aac ttg   3462
Arg Ala Gln Val Asp Pro Leu Val Gln Ser Phe Ser Phe Gln Asn Leu
    1130                1135                1140

cta caa ggc aga atg tac aag atg gtg att cta act cac agt ggg gag   3510
Leu Gln Gly Arg Met Tyr Lys Met Val Ile Val Thr His Ser Gly Glu
1145                1150                1155                1160

ctg tct aat gag tct ttc ata ttt ggt aga aca gtc cca gcc tct gtg   3558
Leu Ser Asn Glu Ser Phe Ile Phe Gly Arg Thr Val Pro Ala Ser Val
                1165                1170                1175

agt cat ctc agg ggg tcc aat cgg aac acg aca gac agc ctt tgg ttc   3606
Ser His Leu Arg Gly Ser Asn Arg Asn Thr Thr Asp Ser Leu Trp Phe
            1180                1185                1190

aac tgg agt cca gcc tct ggg gac ttt gac ttt tat gag ctg att ctc   3654
Asn Trp Ser Pro Ala Ser Gly Asp Phe Asp Phe Tyr Glu Leu Ile Leu
        1195                1200                1205

tat aat ccc aat ggc aca aag aag gaa aac tgg aaa gac aag gac ctg   3702
Tyr Asn Pro Asn Gly Thr Lys Lys Glu Asn Trp Lys Asp Lys Asp Leu
    1210                1215                1220

acg gag tgg cgg ttt caa ggc ctt gtt cct gga agg aag tac gtg ctg   3750
Thr Glu Trp Arg Phe Gln Gly Leu Val Pro Gly Arg Lys Tyr Val Leu
1225                1230                1235                1240

tgg gtg gta act cac agt gga gat ctc agc aat aaa gtc aca gcg gag   3798
Trp Val Val Thr His Ser Gly Asp Leu Ser Asn Lys Val Thr Ala Glu
                1245                1250                1255

agc aga aca gct cca agt cct ccc agt ctt atg tca ttt gct gac att   3846
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Ser Arg Thr Ala Pro Ser Pro Pro Ser Leu Met Ser Phe Ala Asp Ile
          1260                1265                1270

gca aac aca tcc ttg gcc atc acg tgg aaa ggg ccc cca gac tgg aca   3894
Ala Asn Thr Ser Leu Ala Ile Thr Trp Lys Gly Pro Pro Asp Trp Thr
       1275                1280                1285

gac tac aac gac ttt gag ctg cag tgg ttg ccc aga gat gca ctt act   3942
Asp Tyr Asn Asp Phe Glu Leu Gln Trp Leu Pro Arg Asp Ala Leu Thr
   1290                1295                1300

gtc ttc aac ccc tac aac aac aga aaa tca gaa gga cgc att gtg tat   3990
Val Phe Asn Pro Tyr Asn Asn Arg Lys Ser Glu Gly Arg Ile Val Tyr
1305                1310                1315                1320

ggt ctt cgt cca ggg aga tcc tat caa ttc aac gtc aag act gtc agt   4038
Gly Leu Arg Pro Gly Arg Ser Tyr Gln Phe Asn Val Lys Thr Val Ser
                1325                1330                1335

ggt gat tcc tgg aaa act tac agt aaa cca att ttt gga tct gtg agg   4086
Gly Asp Ser Trp Lys Thr Tyr Ser Lys Pro Ile Phe Gly Ser Val Arg
            1340                1345                1350

aca aag cct gac aag ata caa aac ctg cat tgc cgg cct cag aac tcc   4134
Thr Lys Pro Asp Lys Ile Gln Asn Leu His Cys Arg Pro Gln Asn Ser
        1355                1360                1365

acg gcc att gcc tgt tct tgg atc cct cct gat tct gac ttt gat ggt   4182
Thr Ala Ile Ala Cys Ser Trp Ile Pro Pro Asp Ser Asp Phe Asp Gly
    1370                1375                1380

tat agt att gaa tgc cgg aaa atg gac acc caa gaa gtt gag ttt tcc   4230
Tyr Ser Ile Glu Cys Arg Lys Met Asp Thr Gln Glu Val Glu Phe Ser
1385                1390                1395                1400

aga aag ctg gag aaa gaa aaa tct ctg ctc aac atc atg atg cta gtg   4278
Arg Lys Leu Glu Lys Glu Lys Ser Leu Leu Asn Ile Met Met Leu Val
                1405                1410                1415

ccc cat aag agg tac ctg gtg tcc atc aaa gtg cag tcg gcc ggc atg   4326
Pro His Lys Arg Tyr Leu Val Ser Ile Lys Val Gln Ser Ala Gly Met
            1420                1425                1430

act agt gag gtg gtt gaa gac agt act atc aca atg ata gac cgc ccc   4374
Thr Ser Glu Val Val Glu Asp Ser Thr Ile Thr Met Ile Asp Arg Pro
        1435                1440                1445

cct cct cca ccc cca cac att cgt gtg aat gaa aag gat gtg cta att   4422
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Pro Pro Pr  Pr  Pro His Ile Arg Val Asn Glu Lys Asp Val Leu Ile
   1450                1455                1460

agc aag tct tcc atc aac ttt act gtc aac tgc agc tgg ttc agc gac   4470
Ser Lys Ser Ser Ile Asn Phe Thr Val Asn Cys Ser Trp Phe Ser Asp
1465                1470                1475                1480

acc aat gga gct gtg aaa tac ttc aca gtg gtg gtg aga gag gct gat   4518
Thr Asn Gly Ala Val Lys Tyr Phe Thr Val Val Val Arg Glu Ala Asp
                1485                1490                1495

ggc agt gat gag ctg aag cca gaa cag cag cac cct ctc cct tcc tac   4566
Gly Ser Asp Glu Leu Lys Pro Glu Gln Gln His Pro Leu Pro Ser Tyr
            1500                1505                1510

ctg gag tac agg cac aat gcc tcc att cgg gtg tat cag act aat tat   4614
Leu Glu Tyr Arg His Asn Ala Ser Ile Arg Val Tyr Gln Thr Asn Tyr
        1515                1520                1525

ttt gcc agc aaa tgt gcc gaa aat cct aac agc aac tcc aag agt ttt   4662
Phe Ala Ser Lys Cys Ala Glu Asn Pro Asn Ser Asn Ser Lys Ser Phe
    1530                1535                1540

aac att aag ctt gga gca gag atg gag agc tta ggt gga aaa cgc gat   4710
Asn Ile Lys Leu Gly Ala Glu Met Glu Ser Leu Gly Gly Lys Arg Asp
1545                1550                1555                1560

ccc act cag caa aaa ttc tgt gat gga cca ctg aag cca cac act gcc   4758
Pro Thr Gln Gln Lys Phe Cys Asp Gly Pro Leu Lys Pro His Thr Ala
                1565                1570                1575

tac aga atc agc att cga gct ttt aca cag ctc ttt gat gag gac ctg   4806
Tyr Arg Ile Ser Ile Arg Ala Phe Thr Gln Leu Phe Asp Glu Asp Leu
            1580                1585                1590

aag gaa ttc aca aag cca ctc tat tca gac aca ttt ttt tct tta ccc   4854
Lys Glu Phe Thr Lys Pro Leu Tyr Ser Asp Thr Phe Phe Ser Leu Pro
        1595                1600                1605

atc act att gaa tca gag ccc ttg ttt gga gct att gaa ggt gtg agt   4902
Ile Thr Thr Glu Ser Glu Pro Leu Phe Gly Ala Ile Glu Gly Val Ser
    1610                1615                1620

gct ggt ctg ttt tta att ggc atg cta gtg gct ggt gtt gcc tta ttg   4950
Ala Gly L u Phe Leu Ile Gly Met Leu Val Ala Val Val Ala Leu Leu
1625                1630                1635                1640

atc tgc aga cag aaa gtg agc cat ggt cga gaa aga ccc tct gcc cgt   4998
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Ile Cys Arg Gln Lys Val Ser His Gly Arg Glu Arg Pro Ser Ala Arg
                1645                1650                1655

ctg agc att cgt agg gat cga cca tta tct gtc cac tta aac ctg ggc   5046
Leu Ser Ile Arg Arg Asp Arg Pro Leu Ser Val His Leu Asn Leu Gly
            1660                1665                1670

cag aaa ggt aac cgg aaa act tct tgt cca ata aaa ata aat cag ttt   5094
Gln Lys Gly Asn Arg Lys Thr Ser Cys Pro Ile Lys Ile Asn Gln Phe
        1675                1680                1685

gaa ggg cat ttc atg aag cta cag gct gac tcc aac tac ctt cta tcc   5142
Glu Gly His Phe Met Lys Leu Gln Ala Asp Ser Asn Tyr Leu Leu Ser
    1690                1695                1700

aag gaa tac gag gag tta aaa gac gtg ggc cga aac cag tca tgt gac   5190
Lys Glu Tyr Glu Glu Leu Lys Asp Val Gly Arg Asn Gln Ser Cys Asp
1705                1710                1715                1720

att gca ctc ttg ccg gag aat aga ggg aaa aat cga tac aac aat ata   5238
Ile Ala Leu Leu Pro Glu Asn Arg Gly Lys Asn Arg Tyr Asn Asn Ile
                1725                1730                1735

ttg ccc tat gat gcc acg cga gtg aag ctc tcc aat gta gat gat gat   5286
Leu Pro Tyr Asp Ala Thr Arg Val Lys Leu Ser Asn Val Asp Asp Asp
            1740                1745                1750

ccc tgc tct gac tac atc aat gcc agc tac atc cct ggc aac aac ttc   5334
Pro Cys Ser Asp Tyr Ile Asn Ala Ser Tyr Ile Pro Gly Asn Asn Phe
        1755                1760                1765

aga aga gaa tac att gtc act cag gga ccg ctt cct ggc acc aag gat   5382
Arg Arg Glu Tyr Ile Val Thr Gln Gly Pro Leu Pro Gly Thr Lys Asp
    1770                1775                1780

gac ttc tgg aaa atg gtg tgg gaa caa aac gtt cac aac atc gtc atg   5430
Asp Phe Trp Lys Met Val Trp Glu Gln Asn Val His Asn Ile Val Met
1785                1790                1795                1800

gtg acc cag tgt gtt gag aag ggc cga gta aag tgt gac cat tac tgg   5478
Val Thr Gln Cys Val Glu Lys Gly Arg Val Lys Cys Asp His Tyr Trp
                1805                1810                1815

cca gcg gac cag gat tcc ctc tac tat ggg gac ctc atc ctg cag atg   5526
Pro Ala Asp Gln Asp Ser Leu Tyr Tyr Gly Asp Leu Ile Leu Gln Met
            1820                1825                1830

ctc tca gag tcc gtc ctg cct gag tgg acc atc cgg gag ttt aag ata   5574
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Leu Ser Glu Ser Val Leu Pro Glu Trp Thr Ile Arg Glu Phe Lys Ile
      1835                1840                1845

tgc ggt gag gaa cag ctt gat gca cac aga ctc atc cgc cac ttt cac   5622
Cys Gly Glu Glu Gln Leu Asp Ala His Arg Leu Ile Arg His Phe His
   1850                1855                1860

tat acg gtg tgg cca gac cat gga gtc cca gaa acc acc cag tct ctg   5670
Tyr Thr Val Trp Pro Asp His Gly Val Pro Glu Thr Thr Gln Ser Leu
1865                1870                1875                1880

atc cag ttt gtg aga act gtc agg gac tac atc aac aga agc ccg ggt   5718
Ile Gln Phe Val Arg Thr Val Arg Asp Tyr Ile Asn Arg Ser Pro Gly
                1885                1890                1895

gct ggg ccc acc gtg gtg cac tgc agt gct ggt gtg ggt agg act gga   5766
Ala Gly Pro Thr Val Val His Cys Ser Ala Gly Val Gly Arg Thr Gly
            1900                1905                1910

acc ttt att gca ttg gac cga atc ctc cag cag tta gac tcc aaa gac   5814
Thr Phe Ile Ala Leu Asp Arg Ile Leu Gln Gln Leu Asp Ser Lys Asp
        1915                1920                1925

tct gtg gac att tat gga gca gtg cac gac cta aga ctt cac agg gtt   5862
Ser Val Asp Ile Tyr Gly Ala Val His Asp Leu Arg Leu His Arg Val
    1930                1935                1940

cac atg gtc cag act gag tgt cag tat gtc tac cta cat cag tgt gta   5910
His Met Val Gln Thr Glu Cys Gln Tyr Val Tyr Leu His Gln Cys Val
1945                1950                1955                1960

aga gat gtc ctc aga gca aga aag cta cgg agt gaa caa gaa aac ccc   5958
Arg Asp Val Leu Arg Ala Arg Lys Leu Arg Ser Glu Gln Glu Asn Pro
                1965                1970                1975

ttg ttt cca atc tat gaa aat gtg aat cca gag tat cac aga gat cca   6006
Leu Phe Pro Ile Tyr Glu Asn Val Asn Pro Glu Tyr His Arg Asp Pro
            1980                1985                1990

gtc tat tca agg cat tgagaaatgta cctgaagagc tcctggataa aaattactca   6061
Val Tyr Ser Arg His
        1995

ctgtgtgatt tgct                                                   6075

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Leu Gln Thr Gly Leu Ala Glu Pro Glu Arg Cys Asn Phe Thr Leu Ala
            20                  25                  30

Glu Ser Lys Ala Ser Ser His Ser Val Ser Ile Gln Trp Arg Ile Leu
        35                  40                  45

Gly Ser Pro Cys Asn Phe Ser Leu Ile Tyr Ser Ser Asp Thr Leu Gly
    50                  55                  60

Ala Ala Leu Cys Pro Thr Phe Arg Ile Asp Asn Thr Thr Tyr Gly Cys
65                  70                  75                  80

Asn Leu Gln Asp Leu Gln Ala Gly Thr Ile Tyr Asn Phe Lys Ile Ile
                85                  90                  95

Ser Leu Asp Glu Glu Arg Thr Val Val Leu Gln Thr Asp Pro Leu Pro
            100                 105                 110

Pro Ala Arg Phe Gly Val Ser Lys Glu Lys Thr Thr Ser Thr Gly Leu
        115                 120                 125

His Val Trp Trp Thr Pro Ser Ser Gly Lys Val Thr Ser Tyr Glu Val
    130                 135                 140

Gln Leu Phe Asp Glu Asn Asn Gln Lys Ile Gln Gly Val Gln Ile Gln
145                 150                 155                 160

Glu Ser Thr Ser Trp Asn Glu Tyr Thr Phe Phe Asn Leu Thr Ala Gly
                165                 170                 175

Ser Lys Tyr Asn Ile Ala Ile Thr Ala Val Ser Gly Gly Lys Arg Ser
            180                 185                 190

Phe Ser Val Tyr Thr Asn Gly Ser Thr Val Pro Ser Pro Val Lys Asp
        195                 200                 205

Ile Gly Ile Ser Thr Lys Ala Asn Ser Leu Leu Ile Ser Trp Ser His
    210                 215                 220

Gly Ser Gly Asn Val Glu Arg Tyr Arg Leu Met Leu Met Asp Lys Gly
225                 230                 235                 240
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Ile Leu Val His Gly Gly Val Val Asp Lys His Ala Thr Ser Tyr Ala
245 250 255

Phe His Gly Leu Ser Pro Gly Tyr Leu Tyr Asn Leu Thr Val Met Thr
260 265 270

Glu Ala Ala Gly Leu Gln Asn Tyr Arg Trp Lys Leu Val Arg Thr Ala
275 280 285

Pro Met Glu Val Ser Asn Leu Lys Val Thr Asn Asp Gly Ser Leu Thr
290 295 300

Ser Leu Lys Val Lys Trp Gln Arg Pro Pro Gly Asn Val Asp Ser Tyr
305 310 315 320

Asn Ile Thr Leu Ser His Lys Gly Thr Ile Lys Glu Ser Arg Val Leu
325 330 335

Ala Pro Trp Ile Thr Glu Thr His Phe Lys Glu Leu Val Pro Gly Arg
340 345 350

Leu Tyr Gln Val Thr Val Ser Cys Val Ser Gly Glu Leu Ser Ala Gln
355 360 365

Lys Met Ala Val Gly Arg Thr Phe Pro Asp Lys Val Ala Asn Leu Glu
370 375 380

Ala Asn Asn Asn Gly Arg Met Arg Ser Leu Val Val Ser Trp Ser Pro
385 390 395 400

Pro Ala Gly Asp Trp Glu Gln Tyr Arg Ile Leu Leu Phe Asn Asp Ser
405 410 415

Val Val Leu Leu Asn Ile Thr Val Gly Lys Glu Glu Thr Gln Tyr Val
420 425 430

Met Asp Asp Thr Gly Leu Val Pro Gly Arg Gln Tyr Glu Val Glu Val
435 440 445

Ile Val Glu Ser Gly Asn Leu Lys Asn Ser Glu Arg Cys Gln Gly Arg
450 455 460

Thr Val Pro Leu Ala Val Leu Gln Leu Arg Val Lys His Ala Asn Glu
465 470 475 480

Thr Ser Leu Ser Ile Met Trp Gln Thr Pro Val Ala Glu Trp Glu Lys
485 490 495

Tyr Ile Ile Ser Leu Ala Asp Arg Asp Leu Leu Leu Ile His Lys Ser
500 505 510

Leu Ser Lys Asp Ala Lys Glu Phe Thr Phe Thr Asp Leu Val Pro Gly
515 520 525

Arg Lys Tyr Met Ala Thr Val Thr Ser Ile Ser Gly Asp Leu Lys Asn
530 535 540

Ser Ser Ser Val Lys Gly Arg Thr Val Pro Ala Gln Val Thr Asp Leu
545 550 555 560

His Val Ala Asn Gln Gly Met Thr Ser Ser Leu Phe Thr Asn Trp Thr
565 570 575

Gln Ala Gln Gly Asp Val Glu Phe Tyr Gln Val Leu Leu Ile His Glu
580 585 590

Asn Val Val Ile Lys Asn Glu Ser Ile Ser Ser Glu Thr Ser Arg Tyr
595 600 605

Ser Phe His Ser Leu Lys Ser Gly Ser Leu Tyr Ser Val Val Val Thr
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Thr Val Ser Gly Gly Ile Ser Ser Arg Gln Val Val Val Glu Gly Arg
625 630 635 640

Thr Val Pro Ser Ser Val Ser Gly Val Thr Val Asn Asn Ser Gly Arg
645 650 655

Asn Asp Tyr Leu Ser Val Ser Trp Leu Val Ala Pro Gly Asp Val Asp
660 665 670

Asn Tyr Glu Val Thr Leu Ser His Asp Gly Lys Val Val Gln Ser Leu
675 680 685

Val Ile Ala Lys Ser Val Arg Glu Cys Ser Phe Ser Ser Leu Thr Pro
690 695 700

Gly Arg Leu Tyr Thr Val Thr Ile Thr Thr Arg Ser Gly Lys Tyr Glu
705 710 715 720

Asn His Ser Phe Ser Gln Glu Arg Thr Val Pro Asp Lys Val Gln Gly
725 730 735

Val Ser Val Ser Asn Ser Ala Arg Ser Asp Tyr Leu Arg Val Ser Trp
740 745 750

Val His Ala Thr Gly Asp Phe Asp His Tyr Glu Val Thr Ile Lys Asn
755 760 765

Lys Asn Asn Phe Ile Gln Thr Lys Ser Ile Pro Lys Ser Glu Asn Glu
770 775 780

Cys Val Phe Val Gln Leu Val Pro Gly Arg Leu Tyr Ser Val Thr Val
785 790 795 800

Thr Thr Lys Ser Gly Gln Tyr Glu Ala Asn Glu Gln Gly Asn Gly Arg
805 810 815

Thr Ile Pro Glu Pro Val Lys Asp Leu Thr Leu Arg Asn Arg Ser Thr
820 825 830

Glu Asp Leu His Val Thr Trp Ser Gly Ala Asn Gly Asp Val Asp Gln
835 840 845

Tyr Glu Ile Gln Leu Leu Phe Asn Asp Met Lys Val Phe Pro Pro Phe
850 855 860

His Leu Val Asn Thr Ala Thr Glu Tyr Arg Phe Thr Ser Leu Thr Pro
865 870 875 880

Gly Arg Gln Tyr Lys Ile Leu Val Leu Thr Ile Ser Gly Asp Val Gln
885 890 895

Gln Ser Ala Phe Ile Glu Gly Phe Thr Val Pro Ser Ala Val Lys Asn
900 905 910

Ile His Ile Ser Pro Asn Gly Ala Thr Asp Ser Leu Thr Val Asn Trp
915 920 925

Thr Pro Gly Gly Gly Asp Val Asp Ser Tyr Thr Val Ser Ala Phe Arg
930 935 940

His Ser Gln Lys Val Asp Ser Gln Thr Ile Pro Lys His Val Phe Glu
945 950 955 960

His Thr Phe His Arg Leu Glu Ala Gly Glu Gln Tyr Gln Ile Met Ile
965 970 975

Ala Ser Val Ser Gly Ser Leu Lys Asn Gln Ile Asn Val Val Gly Arg
980 985 990

Thr Val Pro Ala Ser Val Gln Gly Val Ile Ala Asp Asn Ala Tyr Ser
995 1000 1005

Ser Tyr Ser Leu Ile Val Ser Trp Gln Lys Ala Ala Gly Val Ala Glu
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Arg Tyr Asp Ile Leu Leu Leu Thr Glu Asn Gly Ile Leu Leu Arg Asn
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Thr Ser Glu Pro Ala Thr Thr Lys Gln His Lys Phe Glu Asp Leu Thr
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Pro Gly Lys Lys Tyr Lys Ile Gln Ile Leu Thr Val Ser Gly Gly Leu
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Phe Ser Lys Glu Ala Gln Thr Glu Gly Arg Thr Val Pro Ala Ala Val
1075 1080 1085

Thr Asp Leu Arg Ile Thr Glu Asn Ser Thr Arg His Leu Ser Phe Arg
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Trp Thr Ala Ser Glu Gly Glu Leu Ser Trp Tyr Asn Ile Phe Leu Tyr
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Asn Pro Asp Gly Asn Leu Gln Glu Arg Ala Gln Val Asp Pro Leu Val
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Gln Ser Phe Ser Phe Gln Asn Leu Leu Gln Gly Arg Met Tyr Lys Met
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Val Ile Val Thr His Ser Gly Glu Leu Ser Asn Glu Ser Phe Ile Phe
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Gly Arg Thr Val Pro Ala Ser Val Ser His Leu Arg Gly Ser Asn Arg
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Phe Asp Phe Tyr Glu Leu Ile Leu Tyr Asn Pro Asn Gly Thr Lys Lys
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Glu Asn Trp Lys Asp Lys Asp Leu Thr Glu Trp Arg Phe Gln Gly Leu
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Val Pro Gly Arg Lys Tyr Val Leu Trp Val Val Thr His Ser Gly Asp
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Ser Leu Met Ser Phe Ala Asp Ile Ala Asn Thr Ser Leu Ala Ile Thr
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Trp Lys Gly Pro Pro Asp Trp Thr Asp Tyr Asn Asp Phe Glu Leu Gln
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Lys Ser Glu Gly Arg Ile Val Tyr Gly Leu Arg Pro Gly Arg Ser Tyr
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Gln Phe Asn Val Lys Thr Val Ser Gly Asp Ser Trp Lys Thr Tyr Ser
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Lys Pro Ile Phe Gly Ser Val Arg Thr Lys Pro Asp Lys Ile Gln Asn
345 1350 1355 1360

Leu His Cys Arg Pro Gln Asn Ser Thr Ala Ile Ala Cys Ser Trp Ile
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Pro Pro Asp Ser Asp Phe Asp Gly Tyr Ser Ile Glu Cys Arg Lys Met
1380 1385 1390

Asp Thr Gln Glu Val Glu Phe Ser Arg Lys Leu Glu Lys Glu Lys Ser
1395 1400 1405

Leu Leu Asn Ile Met Met Leu Val Pro His Lys Arg Tyr Leu Val Ser
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Ile Lys Val Gln Ser Ala Gly Met Thr Ser Glu Val Val Glu Asp Ser
425 1430 1435 1440

Thr Ile Thr Met Ile Asp Arg Pro Pro Pro Pro Pro Pro His Ile Arg
1445 1450 1455

Val Asn Glu Lys Asp Val Leu Ile Ser Lys Ser Ser Ile Asn Phe Thr
1460 1465 1470

Val Asn Cys Ser Trp Phe Ser Asp Thr Asn Gly Ala Val Lys Tyr Phe
1475 1480 1485

Thr Val Val Val Arg Glu Ala Asp Gly Ser Asp Glu Leu Lys Pro Glu
1490 1495 1500

Gln Gln His Pro Leu Pro Ser Tyr Leu Glu Tyr Arg His Asn Ala Ser
505 1510 1515 1520

Ile Arg Val Tyr Gln Thr Asn Tyr Ph Ala Ser Lys Cys Ala Glu Asn
1525 1530 1535

Pro Asn Ser Asn Ser Lys Ser Phe Asn Ile Lys Leu Gly Ala Glu Met
1540 1545 1550

Glu Ser Leu Gly Gly Lys Arg Asp Pro Thr Gln Gln Lys Phe Cys Asp
1555 1560 1565

Gly Pro Leu Lys Pro His Thr Ala Tyr Arg Ile Ser Ile Arg Ala Phe
1570 1575 1580

Thr Gln Leu Phe Asp Glu Asp Leu Lys Glu Phe Thr Lys Pro Leu Tyr
585 1590 1595 1600

Ser Asp Thr Phe Phe Ser Leu Pro Ile Thr Thr Glu Ser Glu Pro Leu
1605 1610 1615

Phe Gly Ala Ile Glu Gly Val Ser Ala Gly Leu Phe Leu Ile Gly Met
1620 1625 1630

Leu Val Ala Val Val Ala Leu Leu Ile Cys Arg Gln Lys Val Ser His
1635 1640 1645

Gly Arg Glu Arg Pro Ser Ala Arg Leu Ser Ile Arg Arg Asp Arg Pro
1650 1655 1660

Leu Ser Val His Leu Asn Leu Gly Gln Lys Gly Asn Arg Lys Thr Ser
665 1670 1675 1680

Cys Pro Ile Lys Ile Asn Gln Phe Glu Gly His Phe Met Lys Leu Gln
1685 1690 1695

Ala Asp Ser Asn Tyr Leu Leu Ser Lys Glu Tyr Glu Glu Leu Lys Asp
1700 1705 1710

Val Gly Arg Asn Gln Ser Cys Asp Ile Ala Leu Leu Pro Glu Asn Arg
1715 1720 1725

Gly Lys Asn Arg Tyr Asn Asn Ile Leu Pro Tyr Asp Ala Thr Arg Val
1730 1735 1740

Lys Leu Ser Asn Val Asp Asp Asp Pro Cys Ser Asp Tyr Ile Asn Ala
745 1750 1755 1760

Ser Tyr Ile Pro Gly Asn Asn Phe Arg Arg Glu Tyr Ile Val Thr Gln
1765 1770 1775

Gly Pro Leu Pro Gly Thr Lys Asp Asp Phe Trp Lys Met Val Trp Glu
1780 1785 1790

Gln Asn Val His Asn Ile Val Met Val Thr Gln Cys Val Glu Lys Gly
1795 1800 1805

Arg Val Lys Cys Asp His Tyr Trp Pro Ala Asp Gln Asp Ser Leu Tyr
1810 1815 1820

Tyr Gly Asp Leu Ile Leu Gln Met Leu Ser Glu Ser Val Leu Pro Glu
825 1830 1835 1840

Trp Thr Ile Arg Glu Phe Lys Ile Cys Gly Glu Glu Gln Leu Asp Ala
1845 1850 1855

His Arg Leu Ile Arg His Phe His Tyr Thr Val Trp Pro Asp His Gly
1860 1865 1870

Val Pro Glu Thr Thr Gln Ser Leu Ile Gln Phe Val Arg Thr Val Arg
1875 1880 1885

Asp Tyr Ile Asn Arg Ser Pro Gly Ala Gly Pro Thr Val Val His Cys
1890 1895 1900

Ser Ala Gly Val Gly Arg Thr Gly Thr Phe Ile Ala Leu Asp Arg Ile
905 1910 1915 1920

Leu Gln Gln Leu Asp Ser Lys Asp Ser Val Asp Ile Tyr Gly Ala Val
1925 1930 1935

His Asp Leu Arg Leu His Arg Val His Met Val Gln Thr Glu Cys Gln
1940 1945 1950

Tyr Val Tyr Leu His Gln Cys Val Arg Asp Val Leu Arg Ala Arg Lys
1955 1960 1965

Leu Arg Ser Glu Gln Glu Asn Pro Leu Phe Pro Ile Tyr Glu Asn Val
1970 1975 1980

Asn Pro Glu Tyr His Arg Asp Pro Val Tyr Ser Arg His
985 1990 1995

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/EP 00/03613

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/42 A61K38/46 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
MEDLINE, EPO-Internal, CHEM ABS Data, BIOSIS, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HUANG L ET AL: "GRB2 and SH-PTP2: potentially important endothelial signaling molecules downstream of the TEK/ TIE2 receptor tyrosine kinase." ONCOGENE, (1995 NOV 16) 11 (10) 2097-103., XP002117444 the whole document	1,4,6
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[X] Further documents are listed in the continuation of box C

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 September 2000	19/09/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040 Tx. 31 651 epo nl Fax (+31-70) 340-3016	Muñoz, M

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INTERNATIONAL SEARCH REPORT

International Application No	PCT/EP 00/03613

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